

Human case of West Nile neuroinvasive disease in Portugal, summer 2015

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A case of West Nile virus (WNV) infection was reported in the Algarve region, Portugal, in the first week of September 2015. WNV is known to circulate in Portugal, with occasional reports in horses and birds (2004 to 2011) and very sporadically human cases (in 2004 and in 2010). Here we present the clinical and laboratory aspects related to the first human case of West Nile neuroinvasive disease reported in Portugal.

In September 2015, a case of West Nile neuroinvasive disease was reported in a patient with neurological symptoms by molecular and serological methods at the National Health Institute (INSA), the reference laboratory for the diagnosis of flaviviruses in Portugal. Although all samples were negative for the presence of West Nile virus (WNV) RNA, positive immunofluorescence results were confirmed by virus neutralisation tests meeting the European Union case definition for WNV infections.

Case description

On 21 July 2015 a man in his 70s was assessed in the emergency room of Faro Hospital with a history of six days of high fever ($\geq 38.5^\circ\text{C}$), headache and altered state of consciousness with progressive prostration, drowsiness and lethargy, confusion, gait difficulty and urinary retention. A macular rash was seen on arms and thighs.

The patient, who lives in a rural area in Alcantarilha, Algarve had frequent contact with animals (chickens, pigs, sheep and horses), had not travelled abroad in the previous year and reported that he had never been vaccinated against flaviviruses (yellow fever, tick-borne encephalitis or Japanese encephalitis virus).

The laboratory findings in the hospital showed haemoglobin 12.4 mg/dL (norm: 13–17 g/dL), leukocytes $14.8 \times 10^9/\text{L}$ (norm: $4\text{--}10 \times 10^9/\text{L}$) and C-reactive protein 40 mg/L (norm: < 5 mg/L). Cerebrospinal fluid (CSF) showed 103 cells/ mm^3 (norm: < 6 cells/ mm^3) with a predominance of lymphocytes, 143 mg/dL protein (norm: 15–60 mg/dL) and 58 mg/dL glucose (norm: 40–60 mg/dL).

At the emergency room, viral meningoencephalitis, rickettsial meningoencephalitis or bacterial meningitis diagnosis were considered and the patient was empirically treated with ceftriaxone (2 g, 12/12 h for 14 days) and doxycycline (100 mg, 12/12 h for 7 days). CSF sample molecular analyses were requested for neurotropic viruses (herpes simplex viruses (1 and 2), Varicella zoster virus, Epstein-Barr virus, cytomegalovirus, human herpes virus (6, 7 and 8) and enterovirus) and arboviruses (Toscana virus and WNV). Serological analysis for *Borrelia burgdorferi*, *Coxiella burnetii*, *Brucella* sp., Epstein-Barr virus, Hepatitis B and C viruses, human immunodeficiency virus (1 and 2), *Mycoplasma pneumoniae*, *Rickettsia conorii* and *Treponema pallidum* were also requested. All results became negative except for *C. burnetii* IgG 200 (cut-off: 200), and serological analysis were requested for Toscana virus and WNV.

After 14 days, the patient had a good outcome and was discharged with minimal residual neurological disease, i.e. some slowing in speech and action. Thirty-four days after the onset of symptoms he returned for further evaluation and blood tests, and neurological examination showed full recovery.

Molecular and serological diagnostics

In the National Institute of Health (INSA), a CSF sample taken on day 6 after symptoms onset was negative

for West Nile virus (WNV) in a RT-PCR specific for WNV lineages 1 and 2 [1]. A urine sample collected on day 34 after symptom onset was also negative by real-time RT-PCR [1,2]. Both samples were also negative when tested by generic pan-flavivirus conventional RT-PCR [3].

A serum sample taken on day 16 after symptom onset was tested by an in-house immunofluorescence assay was positive for WNV-specific IgM with a titre of 1,024 (cut-off: 16) and IgG with a titre of 2,048 (cut-off: 32). A second serum sample taken on day 34 after symptom onset showed a WNV-specific IgM titre of 64 and IgG titre of 4,096. Both serum samples were also subjected to immunofluorescence assays for immunoglobulins specific to other flaviviruses, such as yellow fever, tick-borne encephalitis, Dengue, Zika and Japanese encephalitis virus. All were negative for IgM and presented IgG cross-reaction. A WNV-specific IgM antibody response in CSF, a criterion for case confirmation, was not tested due to the lack of available samples after PCR diagnosis.

The serum samples from day 16 and 34 were tested by microneutralisation assay. Replicates of twofold dilutions of the inactivated test sera were incubated with 100 TCID₅₀ of WNV (strain Egypt 101) at 37°C for 1 hour in 96-well microtitre plates. Vero E6 cells were added (2×10^4 /well) and plates were incubated at 37°C under 5% CO₂. Plates were examined microscopically for cytopathic effects at 4 days post addition of cells. Standard positive (West Nile (strain Eg 101) immune mouse ascytis fluid, CDC, Atlanta) and negative sera were included in the assays. Virus used in each run of the test was back-titrated to confirm the validity of the test. Titres were assigned arithmetically as the dilution of serum giving a 50% neutralisation endpoint. Serum neutralising antibody titres of 10 or higher were considered significant. Microneutralisation assays of both serum samples were positive to a titre of 2,560.

Background

WNV is the most widely distributed mosquito-transmitted flavivirus in the world, and the aetiological agent of West Nile fever and West Nile neuroinvasive disease (WNND) [4]. The virus is maintained in nature in enzootic cycles involving ornithophilic mosquitoes, mainly *Culex* species, as primary vectors and some species of birds as primary reservoirs.

WNV transmission via blood transfusion or organ transplantation is a public health threat because WNV disease symptoms are estimated to occur in only about 20% of infected people (of those, less than 1% may develop WNND). Most infections are asymptomatic (80%) and asymptomatic viraemic donors can transmit the virus to immunocompromised or vulnerable recipients [5]. The acknowledgement of the risk of infected blood donations in the affected areas and the emergence of WNV in Europe in the past 10 years prompted

the European Commission to release a preparedness plan for WNV and blood safety in 2012 [6].

WNV is known to circulate in Portugal with frequent detections in horses and birds [7]. INSA performs reference laboratory diagnosis of flaviviruses in Portugal and previously identified a probable human case in 2010 [8], triggering a WNV survey in horses living in the same area. The survey identified two WNV-positive horses [7]. The first confirmed human cases were diagnosed in two tourists in Ireland after a trip to Algarve in 2004 [9]; they had acquired the infection in the proximity of the human case identified this year.

Public health measures

The patient reported here represents the first serum-positive case to date in Portugal. After the communication of the clinical suspicion of a probable case of WNND, clinical, epidemiological and serological surveillance was implemented by the local health authorities in order to assess the possible presence of WNV in susceptible species in the area [10].

Although the detection of viral RNA is an unambiguous prove of WNV infection, it is known to be challenging in patients with symptomatic infections because viraemia can be low or absent at the time of symptom onset [2]. The negative PCR results in CSF and urine were not unexpected seeing as the samples were collected after the beginning of symptoms. The positive immunofluorescence results were confirmed by virus neutralisation test to ascertain the case confirmation according to the European Union case definition for WNV infections [11].

On 3 September, the General Directorate of Veterinary (DGAV; Direção Geral de Alimentação e Veterinária, Ministério da Agricultura e do Mar) reported three new outbreaks in horses in Loulé, Algarve municipality, and so far, four of 82 horses analysed were positive for WNV infection [12].

The Algarve region possesses a large coastal area characterised by marshlands, salt marshes, small islands, dunes and beaches. Several wetlands and bird sanctuaries are present. Fishery, aquaculture and salt extraction are important human activities, as is tourism particularly in summer. A nationwide vector surveillance programme (REVIVE) has covered the Algarve region since 2008 [13].

Conclusion

Veterinary, human and vector surveillance was initiated in the Algarve municipality after the laboratory report of the WNND human case and is still ongoing. This case highlights the essential role of laboratory diagnostics for early detection and implementation of control measures in vector-borne diseases outbreaks.

Conflict of interest

None declared.

Authors' contributions

LZZ: manuscript preparation and molecular diagnosis at INSA; PP: clinical data and laboratory findings in Algarve; LZZ, MJA, SG, TL, PP: serological diagnosis at INSA; MJA, MF: virus neutralisation diagnosis; MJA: laboratory coordination at INSA. All authors collaborated in the work and participated in the final revision of the manuscript.

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